



AMENDMENTS TO THE SPECIFICATION

Please replace the section entitled "DRAWINGS" beginning with paragraph [0030] on page 18 and ending with paragraph [0061] on page 23 with the following section:

DRAWINGS

[0030] Figure 1 shows the attenuation of wild-type *Listeria* DP-L4056 containing OVA antigen as a function of psoralen S-59 concentration (2 J/cm^2 UVA) along with the measurement of OVA antigen presentation to a dendritic cell line. The bacterial log titer and % of antigen presented relative to untreated (data is for 100 *Listeria* per DC 2.4 cell) are plotted vs. nM S-59.

[0031] ~~Figure 2 shows~~ Figures 2A and 2B show the attenuation of wild-type *Listeria* DP-L4056 (2A) and LLO- mutant DP-L4027 (2B) containing OVA antigen as a function of alkylator compound I concentration along with the measurement of OVA antigen presentation to a dendritic cell line. The bacterial log titer and % of antigen presented relative to untreated (data is for 1 *Listeria* per DC 2.4 cell) are plotted vs. μM compound I.

[0032] Figure 3 shows a comparison of the inactivation of wild type *E. coli* to repair deficient mutant CSR 603 (*uvrA recA phr* mutant) as a function of S-59 concentration (2 J/cm^2 UVA). The bacterial log titer is plotted vs. nM S-59 (log scale).

[0033] Figure 4 shows the mean tumor volume as a function of days post implant of B16 OVA tumors into C57Bl/6 mice that are vaccinated at days 3, 7, and 14. The vaccines tested are with and without S-59 treatment.

[0034] Figure 5 shows the percent survival as a function of days post implant of B16 OVA tumors into C57Bl/6 mice that are vaccinated at days 3, 7, and 14. The vaccines tested are with and without S-59 treatment.

[0035] ~~Figure 6 shows~~ Figures 6A and 6B show flow cytometry results showing a population of spleen cells that are TNF- α and IFN- γ positive from mice vaccinated with wild type *Listeria* with and without OVA expression, with and without S-59 UVA treatment (PCT). Figure 6A shows the

population of cells specific for LLO₁₉₀₋₂₁₀. Figure 6B shows the population of cells specific for OVA.

[0036] Figure 7 shows ELISPOT results showing the number of IFN- γ spot forming cells per 2×10^5 spleen cells upon stimulation with either SL8, LLO₁₉₀₋₂₀₁, or LLO₂₉₆₋₃₀₄, from mice vaccinated with the indicated wild type *Listeria* strains with or without S-59 UVA treatment (PCT).

[0037] ~~Figure 8 shows~~ Figures 8A and 8B show the attenuation of *Listeria* strains with and without deletion of uvrAB. The log titer is plotted vs. nM concentration of psoralen S-59 used (6 J/cm^2). Figure 8A, strains DP-L4017 (L461T LLO mutant) and wild type (DP-L4056). Figure 8B, strains DP-L4017 and DP-L4029 ($\Delta actA$).

[0038] ~~Figure 9 shows~~ Figures 9A-D show the attenuation of DP-L4029 ($\Delta actA$) *Listeria* strain containing OVA antigen as a function of psoralen S-59 concentration along with the measurement of OVA antigen presentation to a dendritic cell line. The parent strain (in this case, $\Delta actA$; 9A, 9C) is compared to the strain with a *uvrAB* deletion ($\Delta uvrAB$; 9B, 9D). The bacterial log titer and % of antigen presentation relative to untreated are plotted vs. nM S-59. Figures 9A, 9B, dosed with 0.5 J/cm^2 UVA, washed *Listeria* once, dosed again with 5.5 J/cm^2 UVA, antigen presentation measured at 1 *Listeria* per DC 2.4 cell. Figures 9C, 9D, *Listeria* was grown in the presence of S-59, then dosed with 6 J/cm^2 UVA, antigen presentation measured at 10 *Listeria* per DC 2.4 cell. (Expanded plots of the data are also provided in Figures 9C and 9D.)

[0039] Figure 10 shows polyacrylamide gels of ^{35}S methionine/cysteine incorporated into protein synthesized by S-59/UVA treated *Listeria monocytogenes* strains DP-L4029 ($\Delta actA$) and DP-L4029 *uvrAB* ($\Delta actA\Delta uvrAB$).

[0040] ~~Figure 11 shows~~ Figures 11A and 11B show the ELISPOT assay for spleen cells from mice vaccinated with 59/UVA treated (two methods) *Listeria monocytogenes* strains DP-L4029($\Delta actA$)-OVA or $\Delta actA\Delta uvrAB$ -OVA, stimulated with OVA specific antigen SL8, LLO specific antigens LLO 190 and LLO 296. Figure 11A shows spot forming colonies on plates stimulated with OVA specific antigen, Figure 11B plots the IFN- γ spot forming cells per 2×10^5 spleen cells for all three antigens.

[0041] ~~Figure 12 shows~~ Figures 12A-C show the Intracellular Cytokine Staining (ICS) assay for spleen cells from mice vaccinated with S-59/UVA treated (two methods) *Listeria*

monocytogenes strains DP-L4029(Δ *actA*)-OVA or Δ *actA* Δ *uvrAB*-OVA, stimulated with OVA derived T cell epitope SL8 (12A), LLO specific class II antigen LLO₁₉₀₋₂₀₁ (12B), or LLO specific class I antigen LLO₂₉₆₋₃₀₄ (12C). The S-59/UVA treated *Listeria* are marked “PCT” (stands for photochemical treatment) in the figure.

[0042] ~~Figure 13 shows~~ Figures 13A-B show the number of colony forming units isolated per spleen (13A) or liver (13B) from mice vaccinated with S-59/UVA treated (two methods) *Listeria monocytogenes* strains DP-L4029(Δ *actA*) or Δ *actA* Δ *uvrAB* and challenged with wild type *Listeria monocytogenes* thirty days after vaccination.

[0043] ~~Figure 14 shows~~ Figures 14A-B show the number of colony forming units isolated per spleen (14A) or liver (14B) from mice vaccinated with S-59/UVA treated (grown with psoralen, then UVA treated) *Listeria monocytogenes* strains DP-L4029(Δ *actA*) or DP-L4029 Δ *actA* Δ *uvrAB* (1x, 3x, or 5x vaccination) and challenged with wild type *Listeria monocytogenes* thirty days after vaccination.

[0044] Figure 15 shows the antibody titer of *Listeria* specific antibodies from serum of mice vaccinated with S-59/UVA treated (grown with psoralen, then UVA treated) *Listeria monocytogenes* strains DP-L4029(Δ *actA*) or Δ *actA* Δ *uvrAB* (1x, 3x, or 5x vaccination).

[0045] Figure 16 shows the percent survival (10 days post challenge) of mice vaccinated with S-59/UVA treated (grown with psoralen, then UVA treated) *Listeria monocytogenes* strains DP-L4029(Δ *actA*) or Δ *actA* Δ *uvrAB* (1x, 3x, or 5x vaccination) and challenged with 20 x LD₅₀ or 100 x LD₅₀ wild type *Listeria monocytogenes* thirty days after vaccination.

[0046] Figure 17 shows the results of an ICS assay for spleen cells from mice vaccinated with S-59/UVA treated (grown with psoralen, then UVA treated) *Listeria monocytogenes* strains DP-L4029(Δ *actA*)-OVA AH1A5 or Δ *actA* Δ *uvrAB*-OVA AH1A5, stimulated with antigens LLO91, AH1, AH1A5, or cells P815 or CT26 cells.

[0047] ~~Figure 18 shows~~ Figures 18A-B show the results of an ELISPOT assay showing plates with spot forming colonies for spleen cells from mice vaccinated with S-59/UVA treated (grown with psoralen, then UVA treated) *Listeria monocytogenes* strains DP-L4029(Δ *actA*)-OVA AH1A5 or Δ *actA* Δ *uvrAB*-OVA AH1A5, stimulated with AH1A5 (18A) or AH1 (18B) antigen.

[0048] ~~Figure 19 shows Figures 19A-C show~~ lungs from mice with established CT26 lung tumors given a therapeutic vaccination with S-59/UVA treated DP-L4029, with or without a $\Delta uvrAB$ mutation (19A). The number of lung metastases are plotted for each vaccine strain (19B). The survival of the remaining mice is plotted in Figure 19C.

[0049] ~~Figure 20 shows Figures 20A-20D show~~ mice with established CT26 tumors were given therapeutic vaccination with *Listeria monocytogenes* $\Delta actA$, $\Delta actA$ AH1-A5, $\Delta actA\Delta uvrAB$ AH1-A5 and $\Delta actA\Delta inlB$ AH1-A5. The $\Delta uvrAB$ strain was either no treatment, heat-killed (HK) or S-59 UVA (PCT) treated. The lungs harvested from a subset of the mice are shown in Figure 20A, with the number of lung metastases in each group plotted in Figure 20B. Survival of the remaining mice is plotted in Figure 20C (parent strain) and 20D ($\Delta uvrAB$ strain).

[0050] Figure 21A shows fluorescent microscopy images of DC 2.4 cells infected by wild type *Listeria monocytogenes* *uvrAB* mutant that has been S-59/UVA treated, showing merged image (both *Listeria* and actin positive) and Rhodamine image (only actin positive). Figure 21B is a plot of the percentage of the *Listeria monocytogenes* that is in the cytoplasm for wild type and $\Delta uvrAB$ strains (live, heat-killed or S-59 UVA treated) compared to LLO⁺.

[0051] Figure 22 shows a negative image photomicrograph of Gram stained *Listeria monocytogenes* wild-type and $\Delta uvrAB$ strains that have been S-59/UVA treated.

[0052] ~~Figure 23 shows Figures 23A-C show~~ the target cell populations following injection into mice vaccinated with the indicated *Listeria* strains or vehicle control. The reduced levels of antigen-specific target cells relative to non-specific target cells indicates *in vivo* cytotoxicity of T cells in response to the vaccination. Figure 23A shows results for AH1-A5 expressing vaccines with vaccination at days 0 (also 1 and 2 for S-59 UVA treated strains). (The top row in 23A and 23B shows results for mice vaccinated with the indicated vaccines for AH1 target cells. The bottom row shows results for mice vaccinated with the indicated vaccines for AH1-A5 target cells.) Figure 23B has a repeat vaccination at day 14 (15 and 16 for S-59 UVA treated) and Figure 23C looks at an OVA specific response.

[0053] Figure 24 shows the attenuation of *Bacillus anthracis* Sterne strain with and without deletion of *uvrAB*. The log titer is plotted vs. nM concentration of psoralen S-59 present during growth and UVA irradiation (6 J/cm²).

[0054] Figure 25 shows *Listeria uvrAB* are more susceptible to S-59/UVA light inactivation. *Listeria* were grown to mid-log phase, washed in PBS, incubated for 5 min with varying concentrations of S-59 and illuminated at 2.1 J/cm² of UVA light. The viability of *Listeria* was assessed by growth on BHI agar plates. (A) Representative BHI agar plates of *Listeria* treated at 100 nM S-59. Heat-killed *Listeria* served as control; (B) Viability of *Listeria* treated at varying concentrations of S-59 to form colonies on BHI agar plates.

[0055] Figure 26 shows that S-59/UVA treated, non-viable *Listeria uvrAB* retain their metabolic activity and the expression of their genomic repertoire. (A) Metabolic activity determined in a MTT assay of S-59/UVA inactivated *Listeria* uvrAB. Live and heat-killed *Listeria* uvrAB served as control; (B) Quantification of the metabolic activity of inactivated *Listeria* uvrAB strain determined in a MTT assay.

[0056] Figure 27 shows that fully inactivated *Listeria uvrAB* retain their capacity to infect DC and to escape from the phagolysosome. The murine DC line, DC2.4, grown on coverslips was infected at an MOI of 1 for 30 min at 37°C. Extracellular bacteria were carefully removed by several washes and infected cells were incubated for 5 hrs at 37°C in the presence of gentamicin to prevent growth of extracellular bacteria. DC2.4 cells were fixed with 3.5% formaldehyde and then stained with rabbit anti-*Listeria* antibody, detected with a goat-anti-rabbit FITC secondary antibody. Actin was detected with Phalloidin-rhodamine and the nucleus was visualized using DAPI.

[0057] Figure 28 shows that fully inactivated *Listeria uvrAB* efficiently load antigen into the MHC class I pathway of murine bone marrow-derived DC (BM-DC). Day 5 BM-DC were infected with a MOI of 100 for 30 min at 37°C. Extracellular bacteria were removed by several washes. Infected BM-DC were co-incubated with B3Z overnight and activation was determined by hydrolysis of the chromogenic substrate CPRG (absorbance).

[0058] ~~Figure 29 shows Figures 29A-C show~~ *Listeria* infected human immature monocyte-derived DC upregulate activation (29A) and maturation markers (29B) as well as secrete pro-inflammatory cytokines (29C). DCs were infected with *Listeria* at different MOI for 1 hour. Infected DCs were cultured for additional 24 hours in the presence of gentamicin to prevent the growth of extracellular bacteria. Phenotypic changes were determined by flow cytometry.

Cytokine levels were determined from cell supernatants using the Cytometric bead array kit (Pharmingen).

[0059] ~~Figure 30 shows~~ Figures 30A-B show that S-59/UVA inactivated *Listeria uvrAB* OVA induce OVA-specific immunity *in vivo*. Female C57BL/6 mice were administered intravenously with 1×10^8 CFU of S-59/UVA inactivated *Listeria uvrAB* OVA. The S-59/UVA inactivated parent *Listeria* strain and heat-killed *Listeria* served as control. Seven days later, spleens were harvested and OVA-specific CD8+ T cell responses were assessed by IFN- γ ELISPOT. (A) Representative ELISPOT wells are shown; (B) OVA-specific immunity assessed by ELISPOT. Spleen cells of vaccinated mice were cultured with or without OVA257-264 peptide.

[0060] Figure 31 shows the primary amino acid sequence of the heterologous antigen LLO-OVA/PR3 (SEQ ID NO:48). The figure also shows the OVA H-2 K^b epitope (SEQ ID NO:49) and the PR3 HLA A-2 restricted class I epitope (a.k.a. PR1) (SEQ ID NO:50).

[0061] Figure 32 shows the compound 4'-(4-amino-2-oxa)butyl-4,5',8-trimethylpsoralen (S-59).